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Hypholoma tuberosum, a new representative of the Czech and Central-European mycobiota

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Abstract — *Hypholoma tuberosum* (*Basidiomycetes*, *Strophariaceae*) has been found in the Czech Republic for the first time, and this collection also represents the first well-documented record in Central Europe. Detailed macro- and microscopic descriptions are given and its world distribution is summarised. The sequencing of ITS and LSU region of nuclear ribosomal DNA confirmed the identity of the species.

Key words — *Basidiomycota*, taxonomy, ribosomal DNA

Introduction

During the excursion to the Hostýnské vrchy hills (Czech Republic, NE Moravia) organised by the second author, an interesting, brightly coloured fungus belonging to *Strophariaceae* was found. It was preliminarily identified as *Hypholoma tuberosum*. However, in 2007, it was collected without a sclerotium and so the species was not fully verified. Collection of many sclerotia during the 2008 season definitely confirmed our earlier identification. Our identification was also supported by the sequencing of ITS region of nuclear ribosomal DNA.

Material and methods

Macroscopic description based on fresh basidiocarps is made by the first author. Microscopic features are described from dried material mounted in

H_2O, c. 3 % KOH, Melzer's reagent and Congo Red using an Olympus BX-50 light microscope with a magnification of ×1000. For basidiospores, the factors E (quotient of length and width in any one spore) and Q (mean of E-values) are used. Authors of fungal names are cited according to Kirk & Ansell (1992) and colour abbreviations follow Kornerup & Wanscher (1983). Herbarium specimens are preserved in the herbarium of the Moravian Museum, Dept. of Botany, Brno, Czech Republic (BRNM).

DNA was extracted from the dried fungal material, using PowerSoil™ DNA Isolation Kit (Mo-Bio). The DNA was amplified with PCR, using the Mastercycler® ep thermocycler (Eppendorf). The PCR amplifications were performed according to Tomšovský et al. (2006), the purification of PCR products and sequencing were done according to Tomšovský & Ryvarden (2008).

Results

Hypholoma tuberosum Redhead & Kroeger, Mycotaxon 29: 457. 1987. Figs 1–2.

Syn. *Psilocybe tuberosa* (Redhead & Kroeger) Walleyn, Sterbeeckia 18: 11. 1998.

Description of collected basidiocarps

Basidiocarps single or in small groups (Fig. 1). Pileus 15–45 mm broad, (broadly) conical, with ± obtuse, often distinct umbo at centre, slightly depressed around it, with inflexed to involute, then straight margin, sparsely radially squamulose in external half, especially at margin when young, soon glabrescent, entirely glabrous when old, connected with stipe by greyish or dirty whitish arachnoid velum, later ± without remnants at margin, never translucently striate, subviscid when moist, greyish orange to brownish orange (6B6–7, 6–7C7), paler, sometimes almost whitish towards margin. Lamellae moderately close, L = 28–32, l = 4–5, emarginate and attached with short tooth, greyish brown (± 6–7E2–3), paler when young, with whitish or white, finely denticulate and pubescent edge; yellow colour translucent among lamellae and lamellae becoming yellowish close to margin in old specimens. Stipe 25–55 × 2.5–4 mm, cylindrical, slightly broadened at apex, fusoid at base (up to 8 mm) and continuing to up to 60 mm long, towards base tapering pseudorhiza, striate, finely fibrillose and distinctly flocculose at apex, distinctly longitudinally fibrillose towards base, finely fibrillose-squamulose (velar remnants) at base in young specimens, those squamules ascending almost up to annulus in older specimens, whitish at apex, basal squamules concolorous with pileus but paler on greyish orange (5B4–5) ground; with brownish basal fibrils. Annulus in upper 1/3 to 1/5, less distinct, fibrillose, dirty white or greyish, then black from spores, soon only in the form of fibrillose zone. Context rather thick, whitish to yellowish in pileus centre, hollow and dirty whitish in stipe, without

Fig. 1. *Hypholoma tuberosum*. Basidiocarps. Photo: J. Burel (larger photo) and V. Antonín (inset)

distinct smell, taste bitterish or mild with bitter aftertaste. Sclerotium hard, irregularly tuberculate-lobate, up to 50 mm large, dark brown-black when fresh and moist, becoming grey-brown when dry, with homogenous, watery (grey-) yellow-brown context, shallowly under soil surface; mostly 1–2(3) basidiocarps grow from one sclerotium. Spore print purple brown.

Basidiospores 10.5–13(–14) × 5.5–7.0 µm, average = 11.7 × 6.2 µm, E = 1.6–2.1, Q = 1.6–1.8, fusoid, ellipsoid-fusoid, thick-walled (walls up to 0.5(–0.75) µm), with up to 1.5 µm large apical germ pore, smooth; wall brown in KOH. Basidia 27–34 × 8.5–11 µm, 4-spored, clavate, constricted at centre. Basidioles 15–34 × 6.0–11 µm, clavate to utriform, sometimes centrally constricted. Trama hyphae cylindrical to subinflated, ± thin-walled, hyaline, non-dextrinoid, up to 12 µm wide. Lamellae edge sterile. Cheilocystidia 23–40 × 6.5–8.5(–10) µm, fusoid, (sub)lageniform, utriform, rarely clavate, often broadly rostrate, sometimes subcapitate, thin-walled, with frequently thick-walled (up to 1.0 µm) apex, often with gelatinous cap and with crystals there. Chrysocystidia (pleurocystidia) 45–50 × (10–)12–18 µm, fusoid, thin-walled, with central to basal yellow (KOH) and yellow-brown (Melzer´s reagent) contents. Pileipellis a cutis of cylindrical, slightly thick-walled, incrusted (zebroid), up to 10 µm wide hyphae with brightly yellow-brown walls and incrustation in KOH; terminal cells adpressed to erect, clavate, cylindrical, sometimes subcapitate,

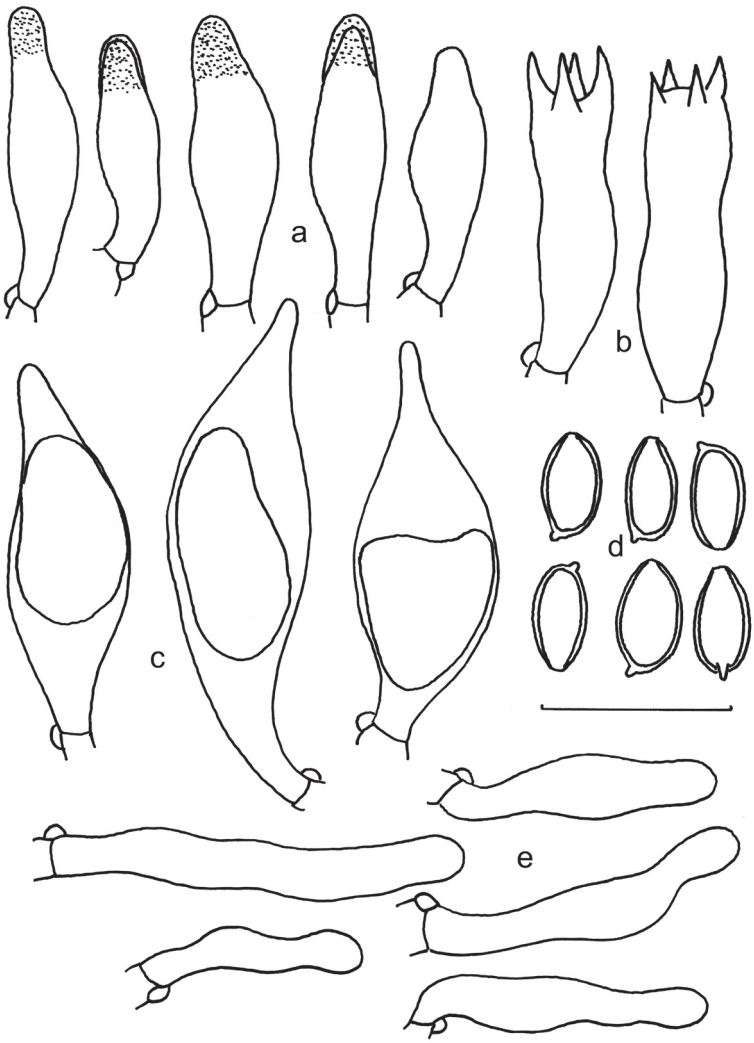

Fig. 2. *Hypholoma tuberosum*.
a. cheilocystidia, b. basidia, c. chrysocystidia, d. basidiospores, e. caulocystidia.
Scale bar = 20 µm.

± thin-walled; subpellis of (sub)globose to ellipsoid, smooth or incrusted cells. Stipitipellis a cutis of cylindrical, parallel, slightly thick-walled, smooth, non-dextrinoid, up to 5.0 µm wide hyphae. Caulocystidia (apex) (24–)30–52 × 5.0–9.0 µm, numerous, cylindrical, clavate, subfusoid, (sub)lageniform,

subutriform, sometimes slightly irregular, subcapitate or rostrate, slightly thick-walled. Annulus of cylindrical, non-dextrinoid, hyaline to pale yellowish (KOH), smooth hyphae. Sclerotium of irregular, branched, thick-walled (up to 1.5 μm), hyaline to pale yellowish, up to 15 μm wide hyphae; terminal cells mostly irregular. Clamp-connections present in all tissues.

Habitat — On soil mixed with wooden chips and sawdust of *Fagus sylvatica* in an insulated open-air place at a depository close to a former charcoal production site along a road (the same place as the type locality of *Bolbitius psittacinus*; Hausknecht et al. 2007).

Locality — Czech Republic, Moravia, Hostýnské vrchy hills, Hošťálková, near Bernátka gamekeeper's lodge, 49º 21' 07" N, 17º 47' 25" E, alt. 550–560 m, 2 Sept. 2007 leg. J. Polčák et al. (Antonín 07.216: BRNM 710220). – Ibid., 31 Aug. 2008 leg. J. Polčák et al. (Antonín 08.178: BRNM 710221).

Molecular data — The obtained ITS and LSU sequences were compared to data deposited in the NCBI database using the BLAST (Altschul *et al.* 1990). The ITS sequence shows 99 % identity to sequence of *H. tuberosum*, accession no. EU682414 (He et al., unpublished) while the LSU sequence is similar (sequence identity of 98–99 %) to those of various strophariod fungi (e.g. *Phaeonematoloma myosotis, Hypholoma marginatum, Stropharia semiglobata, S. umbonescens, Pholiota squarrosa).* Nevertheless, the LSU sequence of *H. tuberosum* was not accessible in the database during the preparation of this manuscript (2 Jan. 2009).

The newly obtained ITS and LSU sequences were deposited to the NCBI under accession numbers FJ461624 and FJ461623 respectively.

Remarks — *Hypholoma tuberosum* is characterised by having an orange-brown pileus, a deeply radicating stem, rather large basidiospores, well-developed cheilo- and chrysocystidia and especially by a well-developed sclerotium.

Characters of our collections agree well with the original description (Redhead & Kroeger 1987) except for slightly longer basidiospores (9.5–11.5 (–12.8) × 5.2–7.0(–7.8) μm in the original description). For the detailed discussion about other *Strophariaceae* species forming sclerotia see Redhead & Kroeger (1987). This species is also included in the key and descriptions of Flora Agaricina Neerlandica (Noordeloos 1999) although it was not known from the Netherlands at that time.

Walleyn (1998) accepted the generic conception of Noordeloos (1995, 1999) and transferred this taxon to the genus *Psilocybe* as *P. tuberosa*.

Hypholoma tuberosum, originally described from British Columbia, Canada, has also been collected on several localities in the U.S.A. (New York, Anonymous 2007b; California, Wood & Stevens 2008; Washington, Oregon, Anonymous 2007a). However, it also has been found in Asia (Japan, four localities, Nagasawa et al. 2000; and China, Sichuan province, He & Liu 2008),

Australasia (Australia, Sydney, Priest & Simpson 1992; and New Zealand, Mid Canterbury, Lincoln, Anonymous 2008). In Europe, it has been collected in two localities in Belgium (Namur and Gent, Noordeloos 1999, Walleyn 1998) for the first time. The third European record is from the Netherlands (Huijser pers. comm.). According to the internet page of the Pilzfreunde Chemnitz e.V. (Germany; http://www.pilzfreunde-chemnitz.de/ pilzkartierung.html), it was also recorded in the Chemnitz region already in 2002. Therefore, this Czech collection represents the fourth (or fifth) European and the first (or second) Central-European locality. It was collected in 2006 for the first time by the second author at the locality mentioned above. Since 2006, the fungus fruits regularly there forming numerous basidiocarps.

This species is connected to ruderal or strongly disturbed biotopes. Originally, it was described from compost piles, rank grass, mulched gardens, and loose soil extensively burrowed by mammals in park in Vancouver, Canada (Redhead & Kroeger 1987). In Japan (Nagasawa et al. 2000) it was found on fertilised soil in a cultivated field, lawns and parks. In Belgium and the Netherlands, it grew on soil mixed with *Populus* wooden chips (Huijser pers. comm., Walleyn 1998). Also the Czech locality is strongly influenced by man. It is a wood depository close to a former charcoal production site along a road where *Hypholoma tuberosum* grows on a forest soil mixed with remnants of wood and sawdust.

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